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INSECTICIDE RESISTANCE OF THE BACTERIA BACILLUS THURINGIENSIS AGAINST BACTROCERA OLEAE UNDER FIELD CONDITIONS

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ABSTRACT

The effect of two isolated Bacillus thuringiensis Bt ,HD 210 and Bt HD128. were tested against the olive fruit pest Bactrocera oleae . data calculated , show that, the Lc50 recoded , 128 and 137 Ug/ml Bactrocera oleae treated with different concentrations of Bt ,HD 210 and Bt HD128, respectively. The experiments carried out under field conditions during two successive seasons 2015 and 2016. The results obtained show that during two panting seasons, 2015 and 2016 the infestations of the olive trees with B. oleae were significantly decreased to 11±4.2 and 30±4.2 individuals after treated with the bacteria Bt ,HD 210 and Bt HD128, respectively as compared to 99±9.9 individuals in the control during season 2015 after 120 days of treatments. During season 2016 the infestations significantly decreased to 17±9.9 and 33±9.4 individuals after treated with the corresponding pathogens as compared to 99±8.4 individuals in the control.

At the harvest time during season 2015, the weight of the olive fruits were significantly increased to 2777± 72.82, and 2831± 12.51 Kg/ feddan in plots treated with Bt, HD 210 and Bt HD128, respectively as compared 2110± 12.51Kg/feddan in the control. During season 2016 the olive fruit significantly increased to Kg/ feddan in plots treated with Bt ,HD 210.

KEY WORDS: Bactrocera oleae ; Bt. Control, Bt ,HD 210, and Bt HD128.

Introduction

Olive (*Olea europaea* L.) has become one of the important economical crops in Egypt. Its cultivated area has been expanded largely in the last decade, particularly in new reclaimed arid areas (Western side of the Nile). Its area reached 49000 Hectares in 2010 (productivity = 6327 Kg/ Hectare) (Mohamed, 2009). Olive tree is subjected to attack by many insect pests that affect yield quality and quantity. Among the most common pest species surveyed in Egypt are: *Bactrocera oleae* (Rossi), *Bactrocera oleae* Bern. and *Ceratitis capitata* (Wied.) (1,2). *B. oleae* is the key pest damaging olive in the world (3) as well as in Egypt (1,2,3,4) it was a native to Mediterranean countries which has 98% of the world's cultivated olive trees (3,4,5). *P. oleae* is one of the most important insect pests of olives in Egypt and other Mediterranean countries. The moth develops three generations per year (3,6,7,8,9). In Egypt the first generation of moths appears in April the female lays its eggs on the flower buds, the newly hatched larvae feed on the buds and flowers (4,10,11,12,13,14,14,16).

Materials and Methods

Laboratory tests:

Insects:

B. oleae adults used in the present work were obtained from laboratory colonies maintained in our laboratory at 25±2°C and 60–65% relative humidity (RH) and 12:12 (L:D) photoperiod. Adults were provided with water and a solid diet consisting of 40% sugar, 10% hydrolyzed yeast, 5% egg yolk.

Microorganisms:

Bacillus thuringiensis Bt ,HD 210*, And Bt HD128,* , were used in this study. The bacterial cultures were maintained on nutrient agar slants at 4°C.

2.3. Bacterial culture media:

The conventional laboratory culture broth, Nutrient broth , was used for culture preparation by mixing 5g peptone and 3g beef extract/ 1 L distilled water. 50 ml of sterile medium was inoculated with one loopful of bacterial strain and incubated under shaking growth conditions on an orbital rotary shaker (125rpm) at 30°C for 72h.

2.4. Effect of the Microbial Control Agents:

Isolated *Bacillus thuringiensis (Bt)* Bt ,HD 210, and *Bt HD128, ;* were used to test their activities on stored insect pests *Bemisia tabaci* , *Bactrocera oleae* adult beetles. The dead larvae of *target insect pest* were collected from the colony. The pathogen were isolated according to [16]. The of Bt the tested concentrations were (500, 250, 125, 63, 32 and 16 ug/ml) (w/v). The rice pots were sprayed by tested concentrations of fungi or Bt and left to dry under laboratory conditions. Control treatment was made by feeding the larvae on untreated rice. The percentages of mortality were counted and calculated according to 50 [17], while LC50 were calculated through probit analysis according to [18]. The experiments were carried under laboratory conditions; 26 ± 2o C and 60- 70% R.H.

Field experiments:

Esraa village- El-Nobaryia region, during the two successive seasons 2015&2016 starting from the first of July till the end of August to evaluate the efficacy of the tested fungi against the target insect pests under field conditions. Three random patches of Olive trees were selected, each comprised 12 trees (12 trees for Bt ,HD 210 and 12 trees for *and Bt HD128,*applications and 12 trees for control) to carry out the field experiment. Bt ,HD 210 and Bt HD*128* were applied, each as a single treatment at the rate of 500 ug/ml. Three applications were made at one week interval at the commencement of the experiment. Treatments were performed at the sunset with a ten litre sprayer. Percentage of infestation/sample was calculated after 20, 50, 90 and 120 days of the application. Each treatment was replicated four times. Four plots were treated with water as control. Random samples of leaves and fruits olives plants were weekly collected from each treatment and transferred to laboratory for examination. The infestation of *B. oleae* were estimated in each case.

Results:

Data in table 1, show that the Lc50 recoded , 128 and 137 Ug/ml after *B. oleae* treated with different concentrations of Bt ,HD 210 and *Bt HD128,*respectively.

During two panting seasons, 2015 and 2016 the infestations of the olive trees with *P. oleae* were significantly decreased to 11±4.2 and 30±4.2 individuals after treated with the bacteria Bt ,HD 210 and *Bt HD128,* respectively as compared to 99±9.9 individuals in the control during season 2015 after 120 days of treatments (table 2). During season 2016 the infestations significantly decreased to 17±9.9 and33±9.4 individuals after treated with the corresponding pathogens as compared to 99±8.4 individuals in the control.

At the harvest time during season 2015, the weight of the olive fruits were significantly increased to . 2777± 72.82 and 2577±55.87 Kg/ feddan in plots treated with Bt ,HD 210 *and Bt HD128,* respectively as compared2110± 12.51Kg/feddan in the control. During season 2016 the olive fruit significantly increased to Kg/ feddan in plots treated with Bt ,HD 210 (Table 3).

Fig 1 show that the infestations of the olive trees with *B. oleae* significantly decreased as compared to control during the experiments days.

These results agree with [19,20,21,22], who proved that the application with bioinsecticides increased the yield and decreased the infestation with insect pests. Also, results were in accordance with [23,24,25,26] who reported that the virulence of *B. bassiana* against *C. capitata* ranged between 8 to 30% and decrease the infestation among the olive fruits. [27,28,29,30] recorded that *C. capitata* mortality ranged between 69 and 78% after bioinsecticides treatments. 31,32,33,34] reported that the fungi *B. bassiana* and *B. brongniartii* application considered the most pathogenic to *C. capitata* causing 97.4 and 85.6% mortality, while *M. anisopliae* cause a highly mortality rates to *C. capitata* and *B. oleae* adults and the rate of larval mortality was 85.2%. In Egypt, [3,4, 32,35,36] reported that the fungi *Lecanicillim lecanii, M. anisopliae* and inter action between *B. bassiana* and *M. anisopliae* are suitable candidates to be used for control of *P. oleae.* [37,38] , controlled cereal aphids with entomopathogenic fungi. They found that the infestation was reduced after fungi applications under laboratory and field conditions. [33,34,36] found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions.

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Table (1): Effect of *pathogens* on the target insect pests under laboratory conditions

Treatments	LC_{50} (Ug/ml)	Slope	Variance	95% Confidence limits
Bt ,HD 210	128	0.01	0.02	166-111
Bt HD128,	137	0.01	0.01	176-110

Table 2. Infested plants with target insect pests after treatment with B.t. under field conditions through out the two seasons

Treatment	Days after treatment	El-Esraa (Nobaryia)	
		Season 2015	Season 2016
Control	20 50 90 120	2 2.1±2.1 21±2.3 78±3.4 99±9.9	25.1±1.4 45±2.5 55±3.4 99±8.4
Bt ,HD 210	20 50 90 120	0±0.0 3±2.2 10±3.4 11±4.2	2±3.9 6±3.4 10±3.7 17±9.9
Bt HD128,	20 50 90 120	0±0.0 2±1.1 16±2.9 30±4.2	3.3±4.7 11±5.4 19±2.3 33±9.4

Table (3): Weight of harvested olive fruits and percentage of yield loss after treatment with the B.t against target insect pests

Treatment	El-Esraa (Nobaryia) during			
	Season 2015		Season 2016	
	Kg/Feddan	% Yield loss	Kg/Feddan	% Yield loss
Control	2110± 12.51	26	2167±70.73	29
Bt ,HD 210	2777± 72.82	--	3080±65.31	-
Bt HD128,	2577±55.87	4	2991±19.13	2
F-value	30.00		30.11	
LSD 5%	83		77	

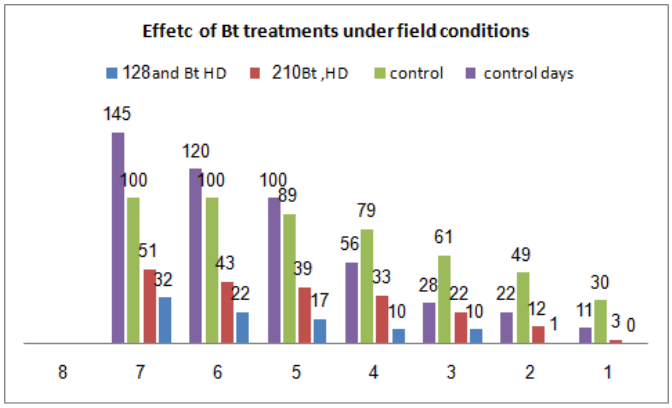

Fig 1. Effect of B.t thuringiensis on B. oleae infestations under field conditions

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